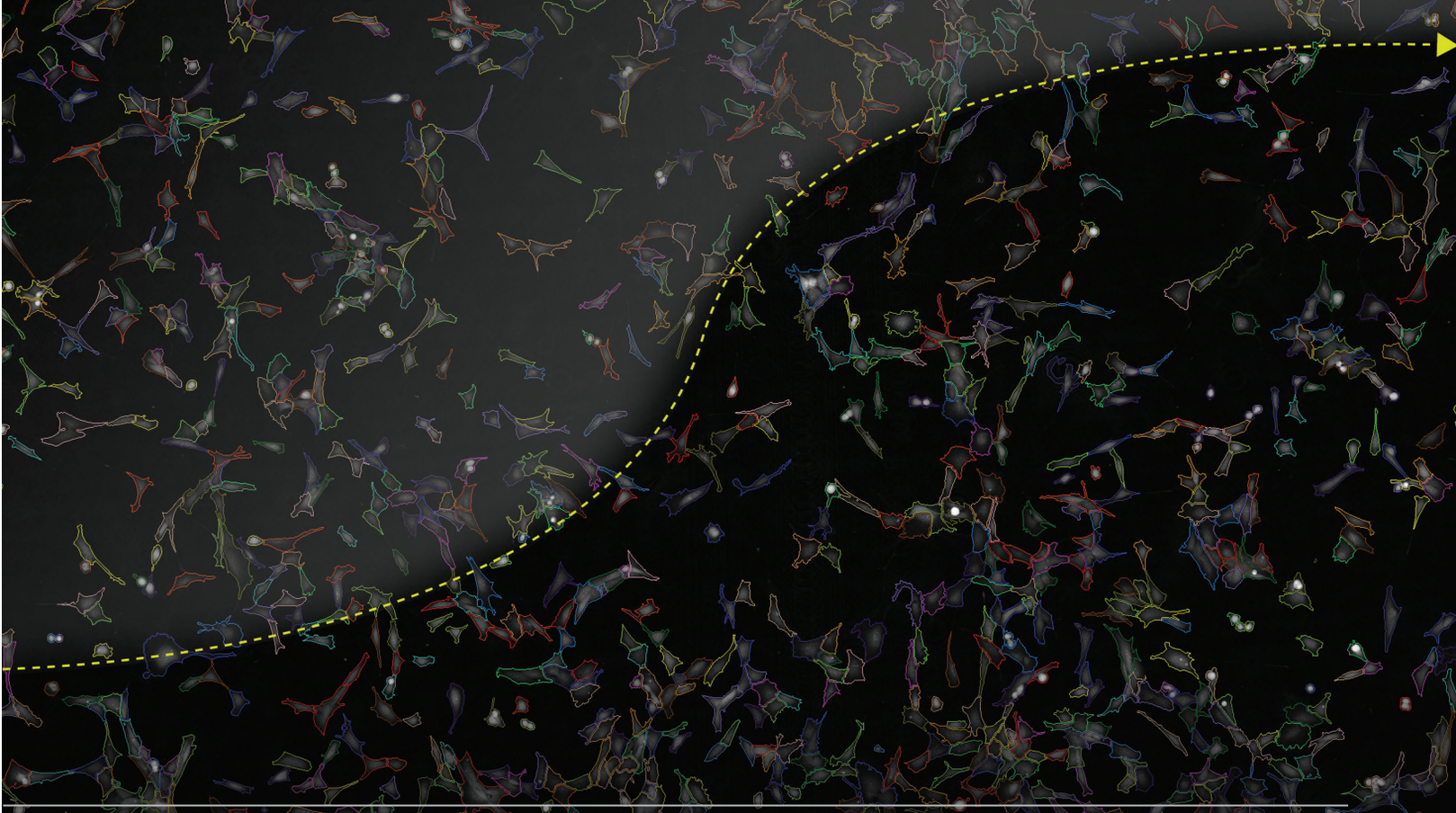


Cell Proliferation assay | Application Note

Real-time cell proliferation

Delve deeper into the dynamics of cell proliferation through non-invasive time-lapse imaging with the Livecyte system

- Measure cell count at every time point
- Time-resolved kinetic information related to rate of cell proliferation
- Directly measure cell proliferation through accurate determination of cell number
- Viable and unperturbed cells allow further downstream assays

phasefocus

Introduction

Cell proliferation is characterised as the increase in the number of cells that results exclusively from the completion of the cell cycle (Pardee, 1989). Changes in proliferation convey defects in cell cycle regulation that are central to cancer pathogenesis (Whitfield et al., 2006). Accordingly, proliferation is used as a metric to assess the effects of candidate pharmaceuticals upon cancerous cells and is an important measure in standard motility, cell activation and cytotoxicity bioassays. In this application note, we show that the Livecyte system can perform single cell automated segmentation and can produce measures of true proliferation.

Methods

MDA-MB-231 or NIH-3T3 cells were seeded into plastic 6-well plates (Corning) at 5 x 10^4 cells/well and allowed to adhere for 24h. For comparison of Livecyte and Vi-CELL proliferation rates, 4x6-well plates seeded with MDA-MB-231 cells were incubated for 0, 6, 24 and 48 h at 37 °C, 5% CO_2. After incubation, three wells of each plate were imaged. The media was removed, cells in each well were washed in PBS and detached with 200 µL 0.25% Trypsin-EDTA for 3 min at 37 °C, quenched with the original media, centrifuged at 300 x g for 5 min along with all PBS and culture media then re-suspended in 500 µL DMEM and run on the Vi-CELL (Beckman Coulter). For the drug-treatment assay, NIH-3T3 cells were treated with 100 nM Staurosporine for 30 min prior to beginning imaging, whilst the control was left untreated.

Results

The Phasefocus Livecyte system was used to produce label free high contrast images over large fields of view, without any image artefacts or stitching (Fig. 1). Due to the label free, high contrast images obtained with the Livecyte system, robust cell segmentation is achieved and consequently the ability to explore the population behaviour down to a single cell level. A total of four 6 well plates were imaged every hour for the following continuous time-periods: 0 h (plate I), 1-6 h (plate II), 7-24 h (plate III), 25-48 h (plate IV). Immediately after image acquisition, the cells were detached and run through a Vi-CELL in order to obtain a cell count for each well via independent means.

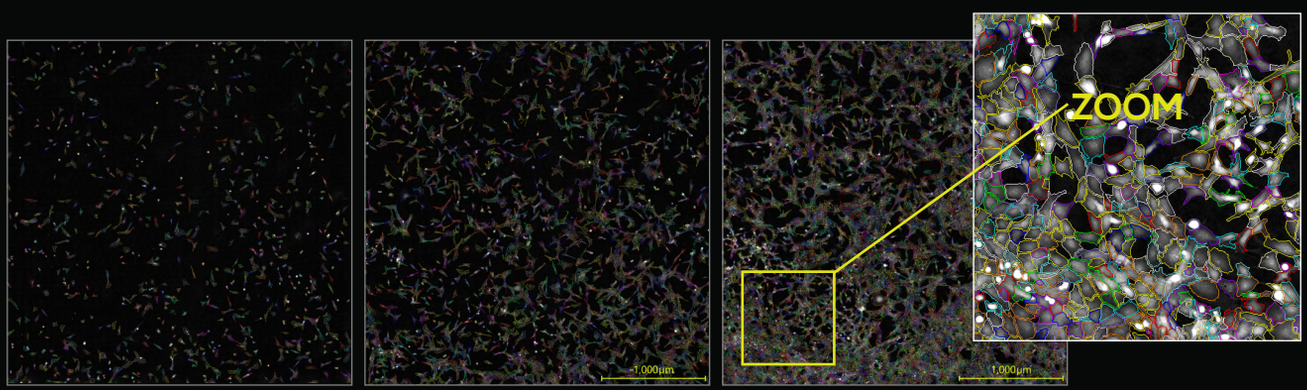


Fig 1: Phase images showing the proliferation of NIH-3T3 cells within a large (3.2 x 3.2 mm) region. Despite the large size of the region, individual cells can be clearly identified, as shown in the digital zoom of the image (yellow box).

Plotting the manual and CAT (Cell Analysis Toolbox) cell counts against one another shows that there is concordance between the values (Fig. 2). The mean difference between measurements is 1.9 cells and 95% of the differences lie between -7.2 and 11.0. This indicates that the automated CAT cell count may overestimate the count by 7 cells or underestimate by up 11 cells according to 95% limits when compared to a manual count of the same region by eye. Note that the median CAT cell count for each region was 95 cells.

Cell counts from the Livecyte and those from the Vi-CELL were plotted against the time at which the counts were taken (i.e. 0, 6, 24 or 48 h). For each technique, the rate of increase in the cell count was estimated by fitting this data with a single exponential model and extracting the gradient from the fit (Fig. 3). No significant difference was revealed through statistical comparison of the gradient values that were derived from each technique; thus indicating that the rate of cell proliferation calculated with the Livecyte is comparable to that measured using an automated cell counter. Changes in the rates of cell proliferation can be useful indicators of the effects of a drug. We also demonstrated the effect of 100 nM Staurosporine on the proliferation of NIH-3T3 cells. Normalised cell counts extracted from phase images of the sample of NIH-3T3 cells that were treated with 100 nM Staurosporine show that the drug has an anti-proliferative effect on the cells when compared to the untreated control (Fig. 4).

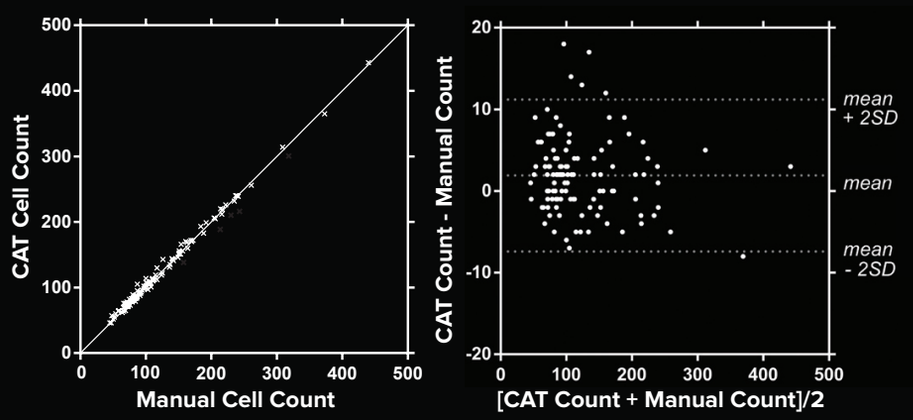


Fig 2: Plot of manual (by eye) and automated (CAT) cell counts for each region imaged (n= 118). To help identify how far one count is from the other, this data is also plotted as the difference versus the mean of CAT and manual counts.

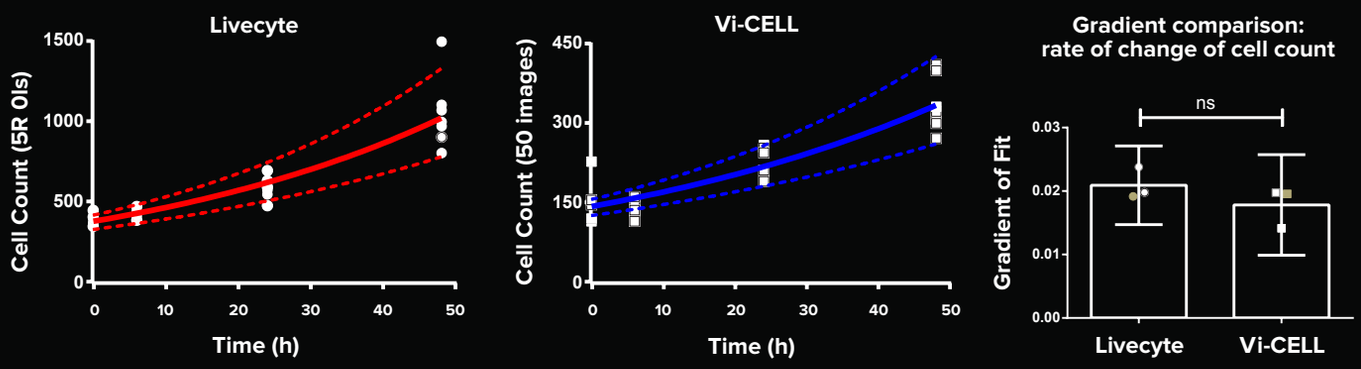
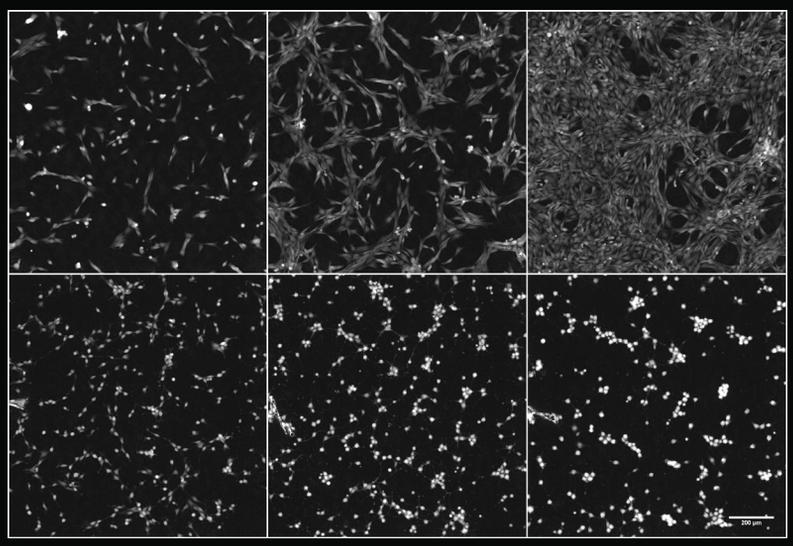


Fig 3: Plots of Livecyte and Vi-CELL MDA-MB-231 cell counts taken from the same wells of 6 well plates incubated for 0, 6, 24 or 48 h. Single exponential fit (solid line); 95% CI (dashed line).



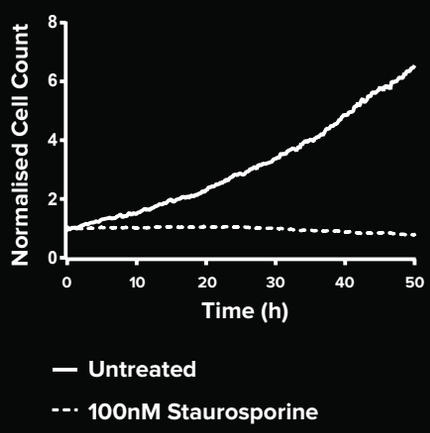


Fig 4: Phase images taken from a 50 h timelapse of NIH-3T3 cells ± staurosporine treatment. The plot shows the rate of proliferation of cells under each treatment condition.

Conclusion

Traditional techniques (phase contrast, brightfield, DIC) do not have ability to robustly segment individual cells and as a consequence, associate cell proliferation measurements with population confluence measurements. However, confluence measurements will be subject to changes in cell morphometry (cell area, cell mass) and as such cannot be a dependable indicator of true cell proliferation. The Livecyte automatic cell count offers an increase in speed of analysis and is accurate to within 10% compared with counting cells manually. Livecyte can provide a continuous count of the cell number. The cells remain viable and unperturbed, which allow further downstream assays to be performed.

References

Pardee, A. B. (1989) G1 events and regulation of cell proliferation. Science, 246, 603-608.
Whitfield, M. L., George, L. K., Grant, G. D. & Perou, C. M. (2006) Common markers of proliferation. Nat Rev Cancer, 6, 99-106.

livecyte

For more information on the benefits of the Livecyte system, to access application notes and for additional product information, please visit: www.phasefocus.com/livecyte

A sample of time-lapse videos can be found at: www.youtube.com/phasefocuslimited

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